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EFFECT OF MAGNESIUM ION CONCENTRATION ON THE PHOTOCHEMICAL FORMATION OF AMINO ACIDS

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Different methods have been proposed by Bahadur and Ranganayaki for the synthesis of amino acids and peptides[1,2]. Recently they have reported the formation of amino acids and peptide in the aqueous mixture of ammonium molybdate, di-ammonium hydrogen phosphate, mineral solution and formaldehyde[3].

Many metals can act as catalyst in the reaction producing compounds of biological interest[4]. Magnesium is one of the metals which is widely distributed in living systems. Magnesium also plays a vital role in plant-animal physiology. Magnesium sulphate markedly inhibited the increase of lactic acid, pyruvic acids and urea in blood or rabbits exposed to high temperature[5]. Magnesium acts as a primary and prevalent factor for preservation of active RNA structure in live green cell. RNA synthsis ceases immediately after withdrawal of magnesium from the medium and resumes rapidly after addition of magnesium to the medium[6]. Magnesium activates DNasa[7] and kinase[8].

Magnesium ions are one of the constituents of the mixture used by Bahadur and Ranganayaki[3], it was decided to study the influence of magnesium ion concentration on the formation of amino acids in the mixture.

The mixture in which influence of magnesium sulphate was studied contain[redacted]ium molybdate, di-ammonium hydrogen phosphate, mineral solut[redacted]ng sodium, potassium, calcium, magnesium, chloride, sulp[redacted]etate and formaldehyde.

EXPERIMENTAL

Ammonium molybdate solution : 4.0 g of ammonium molybdate was dissolved in 100 ml of distilled water.

Di-ammonium hydrogen phosphate solution : 3.0 g of di-ammonium hydrogen phosphate was dissolved in 100 ml of distilled water.

Formaldehyde solution : 36% formaldehyde of B.D.H AnalaR grade has been used.

Mineral solution : Six mineral solutions containing the following substances were prepared :-
(a) 0.02 g of sodium chloride , (b) 0.02 g of potassium sulphate , (c) 0.02 g calcium acetate and (d) 0.00 g, 0.02 g , 0.04 g , 0.06 g, 0.08 g , and 0.10 g magnesium sulphate was added respectively in six flasks No. 1, 2, 3, 4, 5, and 6 respectively. When all the above salts dissolved 0.02 g potassium di-hydrogen orthophosphate was added in each flasks and the volume was raised upto 100 ml with distilled water.

10 ml mineral solution were transferred from flasks No. 1, 2, 3, 4, 5 and 6 into another six flasks No. 1', 2', 3', 4', 5' and 6' respectively which also contained 10 ml ammonium molybdate solution and 20 ml di-ammonium hydrogen phosphate solution. The

flasks containing these mixtures were cotton plugged and sterilized by heating unt[illegible] comes out of the cotton plug. The mixtures were incubat[illegible]r 24 hours and then boiled again as above. The mixtures w[illegible]inally cooled and 10 ml formaldehyde solution were added in e[illegible]sk aseptically.

Another set identical to flasks No. 1', 2', 3', 4', 5' and 6' ~~respextively~~ was prepared and covered with thick black cloth to check the entry of the visible light. The uncovered and covered flasks of two sets were placed in artificial light, at a distance of 0.5 meter from the 100 Watts electric bulb with occasional shaking. After 72 hours exposure the mixtures were taken out aseptically and were examined for sterility by petri-dish technique and was found to be sterile.

The mixtures were subjected to chemical analysis. The detection and identification of amino acids were done by chromatographic technique. The confirmation of amino acids having near R_f values was done simultaneous running of the known amino acids using three running solvent, viz. butanol : acetic acid : water (80 : 20 : 20) ; phenol : water (80 : 20) and alcohol : water (75:25).

Qualitative Analysis of Amino Acids Synthesised in the Mixtures

Containing Dif[redacted]ount of Magnesium Sulphate :

Amount of Magnesium sulphate(g)	[redacted]	Amino acids synthesised in the mixture	Amino acids identified in the hydrolysed mixture
0.00	Light	Glutamic acid	Glycine
		Aspartic acid	Threonine
		Threonine	Glutamic acid
		Serine	Arginine
		Hydroxy proline	Serine
		A few unidentified spots	Tyrosine
0.00	Dark	Histidine	Glycine
		Arginine	Alanine
		Tyrosine	Tyrosine
		Lysine	
		A few unidentified spots	
0.02	Light	Glutamic acid	Glycine
		Serine	Threonine
		Arginine	Aspartic acid
		Glycine	Arginine
		Threonine	
		Aspartic acid	
		A few unidentified spots	
0.02	Dark	Glutamic acid	Threonine
		Arginine	Glycine
		Alanine	Alanine
		Threonine	Aspartic acid

Amount of magnesium sulphate (g)	Condition	Amino Acids synthesised in the mixture	Amino acids identified in the hydrolysed mixture
		ontinued)	
		Aspartic acid	
		Leucine	
		Histidine	
		A few unidentified spots	
0.04	Light	Glutamic acid	Threonine
		Aspartic acid	Glycine
		Alanine	Alanine
		Glycine	Tyrosine
		Arginine	
		Histidine	
		Tyrosine	
		Valine	
		A few unidentified spots	
0.04	Dark	Glutamic acid	Threonine
		Aspartic acid	Glycine
		Threonine	Valine
		Alanine	Alanine
		Glycine	
		Arginine	
		Histidine	
		Valine	
		Tyrosine	
		A few unidentified spots	
0.06	Light	Glutamic acid	Threonine

Amount of magnesium sulphate (g)	Condition	Amino acids synthesised in the mixture	Amino acids identified in the hydrolysed mixture
		(continued)	
		Aspartic acid	Glycine
		Threonine	Alanine
		Alanine	Valine
		Glycine	Serine
		Arginine	
		Tyrosine	
		A few unidentified spots	
0.06	Dark	Glutamic acid	Threonine
		Threonine	Glycine
		Aspartic acid	Alanine
		Glycine	Valine
		Alanine	Serine
		Serine	
		Arginine	
		Tyrosine	
0.08	Light	Glutamic acid	Threonine
		Aspartic acid	Glycine
		Threonine	Alanine
		Alanine	Valine
		Glycine	
		Histidine	
		Tyrosine	
		Serine	
		Arginine	
		A few unidentified spots	

Amount of magnesium sulphate (g)	[illegible]on	Amino acids synthesised in the mixture	Amino acids identified in the hydrolysed mixture
		tinued)	
0.08		Glutamic acid	Threonine
		Threonine	Glycine
		Alanine	Alanine
		Glycine	Valine
		Histidine	
		Tyrosine	
		Argonine	
		A few unidentified spots	
0.10	Light	Glutamic acid	Threonine
		Aspartic acid	Alanine
		Alanine	Glycine
		Threonine	
		Serine	
		Methionine	
		Few unidentified spots	
0.10	Dark	Glutamic acid	Threonine
		Aspartic acid	Glycine
		Alanine	Alanine
		Threonine	
		Serine	
		Tyrosine	
		Methionine	
		A few unidentified spots	

DISCUSSION

A [redacted]mino acids were synthesised in the mixture It was observ[redacted]esium sulphate favours the synthesis of maximum number [redacted]acids. Few spots could not be identified in each case. The sample on hydrolysis with 6N hydrochloric acid showed the presence of some new amino acids but a few amino acids observed in the unhydrolysed sample disappeared which may be due to the decomposition of those amino acids during hydrolysis, particularly when they were present in small quantities.

Glycine, threonine, and alanine had been identified in each case in the hydrolysed sample except in the mixture in which magnesium sulphate was absent. In this case exposed unhydrolysed mixture were found to have glycine and threonine and unexposed hydrolysed sample contained glycine and alanine. Valine was detected on hydrolysis of the exposed and unexposed mixture which contained 0.06 g and 0.08 g of magnesium sulphate. Valine was also detected in the mixture kept in dark containing 0.04 g magnesium sulphate. It was found that magnesium sulphate helps in the formation of arginine within the certain limit of magnesium ion concentration. Arginine was detected in the mixtures containing 0.02 g, 0.04 g, 0.06 g or 0.08 g magnesium sulphate but it was absent in the mixture which had 0.10 g magnesium sulphate. Sulphur containing amino acid viz. methionine was formed only in the exposed mixture containing 0.10 g of magnesium sulphate.

It was also observed that glutamic acid and threonine were synthesised in each case even when magnesium sulphate was absent in the mixture.

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EFFECT OF CALCIUM ION CONCENTRATION ON THE PHOTOCHEMICAL FORMATION OF AMINO ACIDS

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Bahadur [redacted] Ranganayaki have synthesised the amino acids and peptides by various methods[1,2]. They have also reported the formation of amino acids and peptide in the mixture of ammonium molybdate, di-ammonium hydrogen phosphate, mineral solution and formaldehyde in aqueous solution[3]. The mixture contains some of the amino acid in free state while some of them were present in the combined state as peptides.

It is well known that many metals can act as catalyst in the reaction producing compounds of biological interest[4]. Formaldehyde produces large number of sugars in the presence of lime[5]. Chalk ($CaCO_3$) was also used in the mixture whichis involved irradiation of an aqueous solution of formaldehyde and ammonium ion by ultraviolet light from PRK-2 quartz lamp and in which amino acids were produced[6]. Phosphorylase kinase is inactive at pH 6.8, but it can be activated by Ca^{++} ([7]). The amylase activity is increased by the addition of calcium[8]. Calcium activate alkaline phosphatase and nucleotidase in tissue[9].

In view of the importance of Ca^{++} in physiology and abiogenesis as described above and as calcium ions are one of the constituents of the mixture used in this experiments, it was decided

to study the influence of calcium ion concentration in the formation of amino acids in the mixture. The mixture in which the effect of calcium concentration was studied containeds ammonium molybdate, di-ammonium hydrogen phosphate, mineral solution containing sodium potassium, calcium, magnesium, chloride, sulphate and acetate and formaldehyde.

EXPERIMENTAL

Ammonium molybdate solution : 4.0 g ammonium molybdate was dissolved in 100 ml of distilled water.

Di-ammonium hydrogen phosphate solution : 3.0 g di-ammonium hydrogen phosphate was dissolved in 100 ml distilled water.

Formaldehyde solution : 36% formaldehyde B.D.H. AxR AnalaR grade was used for the purpose.

Mineral solution : Six mineral solution containing the following substances were prepared :

(a) 0.02 g sodium chloride ; (b) 0.02 g potassium sulphate ; (c) 0.02 g magnesium sulphate ; (d) 0.01 g , 0.02 g, 0.04 g, 0.06 g, 0.08 g, and 0.10 g calcium acetate was added respectively in the six flasks No.1, 2, 3, 4, 5, and 6 respectively. When all the substance had dissolved 0.02 g potassium di-hydrogen ortho-phosphate was added in each flask and the volume of each solution was made upto 100 ml with distilled water.

10.0 ml mineral solutions were transferred from flask No. 1, 2, 3, 4, 5 and 6 respectively into another flasks No. 1', 2', 3', 4', 5' and 6' respectively which also contained 10.0 ml of 4 percent

ammonium molybdate (w/v) solution and 20.0 ml of 3 percent di-ammonium hydrogen phosphate solution (w/v). All the flasks containing these mixture were cotton plugged and sterilized by boiling. The mixture were incubated at 20°C for 24 hours and then boiled again as above, mixture were then finally cooled and 10.0 ml formaldehyde solution was added to flasks aseptically. The mixtures were kept 0.5 meter below watt electric bulb for exposure. A turbidity started appearing after about an hour. The mixtures were allowed to stand for 72 hours with occasional shaking. The mixtures showed the formation of a little sediment and became light blue.

Another set identical to the flask No. 1', 2', 3', 4', 5' and 6' respectively was prepared and covered with several folds of thick black cloth and kept near the exposed mixture as the control for dark. These mixtures did not show the formation of the particles after 72 hours and remained clear and colourless.

After 72 hours the mixtures were examined for sterility by petri dish technique and was found to be sterile. The detection and identification of amino acid were done by one way and two way paper chromatography technique and ninhydrin dissolved in acetone was used as spraying reagent to develop the chromatograms. The confirmation of amino acids having near R_f values were done by simultaneous running of the known amino acids using three running solvents, viz. butanol: acetic acid : water (80 : 20 : 20) ; phenol : water (80 : 20) and alcohol : water (75 : 25) as running solvents.

Qualitative Analysis of Amino Acids Synthesised in the Mixture Containing Different Amount of Calcium Acetate.

Amount of Calcium acetate used(g)	Condition	Amino acid synthesised in the mixture	Amino acid identified in hydrolysed mixture
0.00		Glutamic acid	Glutamic acid
		Serine	Aspartic acid
		Cystine	Arginine
		A few un-identified spots	Histidine
			Valine
0.00	Dark	Glutamic acid	Valine
		Serine	Serine
		Arginine	Threonine
		Glycine	Tyrosine
		Hydroxy proline	
		Leucine	
		A few unidentified spots	
0.02	Light	Glutamic acid	Aspartic acid
		Serine	Arginine
		Arginine	Threonine
		Glycine	Tyrosine
		Cystine	Alanine
		A few unidentified spots	
0.02	Dark	Glutamic acid	Aspartic acid
		Serine	Arginine
		Cystine	Threonine
		Arginine	Tyrosine

Amount of Calcium acetate used (g)	Condition	Amino acid synthesised in mixture	Amino acid identified in hydrolysed mixture
		(continued)	
		Glycine	Alanine
		Leucine	
		A few unidentified spots	
0.04		Glutamic acid	Tyrosine
		Serine	Alanine
		Threonine	Cystine
		Glycine	Histidine
		Methionine	
		A few unidentified spots	
0.04	Dark	Glutamic acid	Tyrosine
		Serine	Alanine
		Leucine	Cystine
		Glycine	Histidine
		Cystine	
0.06	Light	Glutamic acid	Glutamic acid
		Serine	Serine
		Threonine	Arginine
		Glycine	Aspartic acid
		Arginine	Glycine
		Methionine	Ornithene
		Alanine	
		Aspartic acid	
		Tyrosine	
		Histidine	
		Cystine	

Amount of Calcium acetate used (g)	Condition	Amino acid synthesised in mixture	Amino acid identified in hydrolysed mixture
		(continued)	
		Few unidentified spots	
0.06	[illegible]	Glutamic acid	Aspartic acid
		Serine	Arginine
		Glycine	Glycine
		Aspartic acid	Alanine
		Ornithene	Methionine
		Few unidentified spots	Cystine
0.08	Light	Glutamic acid	Aspartic acid
		Serine	Threonine
		Threonine	Alanine
		Glycine	Cystine
		Alanine	Valine
		Tyrosine	
		Hydroxy proline	
		Cystine	
		Leucine	
		Iso-leucine	
		Few unidentified spots	
0.08	Dark	Glutamic acid	Aspartic acid
		Serine	Threonine
		Threonine	Alanine
		Glycine	Cystine
		Alanine	Valine
		Tyrosine	

Amount of Calcium acetate used (g)	Condition	Amino acid synthesised in mixture	Amino acid identified in hydrolysed mixture
		(continued)	
		Hydroxy proline	
		Cystine	
		Leucine	
		Iso-leucine	
		Few unidentified spots	
0.10	Light	Glutamic acid	Threonine
		Serine	Alanine
		Threonine	Glycine
		Arginine	Methionine
		Hydroxy proline	Valine
		Histidine	Cystine
			Histidine
0.10	Dark	Glutamic acid	Threonine
		Serine	Alanine
		Threonine	Glycine
		Arginine	Methionine
		Glycine	Valine
		Cystine	Histidine
		Methionine	
		Tryptophane	
		Leucine	

DISCUSSION

Various natural amino acid were synthesised in the mixture. it was observed that 0.06 g and 0.08 g calcium acetate favours the

formation of maximum number of amino acid. A few spot could not be identified in each case. However, the sample were hydrolysed with 6N hydrochloric acid in sealed tubes at 100°C for 24 hours. The hydrolysed sample showed presence of a few ne amino acid but a few amino acids observed in the unhydrolysed sample disappeared which may be due to the decom[illegible] of those amino acids during hydrolysis particularly when th[illegible] in smaller quantities.

Conside[illegible] the nature of amino acids synthesised in the mixture , the formation of glutamic acid and serine were observed in each case even when there was no Ca^{++} ion in the mixture. Histidine was detected in the free state in the mixture which contained 0.06 g of calcium acetate. It was also detected in the hydrolysed sample in which 0.04 g and 0.10 g calcium acetate were added. Methionine was observed in the mixture which contained 0.04 g, 0.06 g and 0.1 g calcium acetate. Leucine was synthesised in each sample kept in dark except in the mixture which contain 0.06 g of calcium acetate showing that light has no influence on the formation of leucine.

Hence, it is concluded that Ca^{++} ion help in the formation of a few new amino acids and in general, it increases the quantity of amino acids synthesised upto 0.06 g/100 ml further increase of calcium acetate in the mixture does not increase the amount of amino acid synthesised.

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